8 COLOR TESTS

8.1 Introduction:

8.1.1 Color tests are used as a screening test at the beginning of an analysis. Most are performed on clean porcelain or disposable plastic spot plates; however some may be performed in disposable culture tubes (e.g., Scott's, Tannic Acid).

8.1.2 The test reagent should be added to the plate or tube first, and then the questioned sample. This practice determines if the plate or tube was clean before the analysis.

8.1.3 Several of the listed reagents have more than one recipe listed. Any of the listed, referenced recipes may be utilized in casework and should be reflected in the reagent logbook.

8.1.4 Reagents, indicators and solutions listed in the USP-NF may be used for their published purposes.

8.1.5 Positive color reactions are noted in each of the individual drug sections. These positive reactions may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos). The color observed must be noted for drugs not routinely encountered. Negative reactions may be recorded in a similar fashion. (For Duquenois-Levine results see Section 6.5.1)

8.1.6 Most color test reagents are comprised of strong acids and chemicals requiring careful handling. Appropriate safety precautions should be observed. Refer to MSDS's for storage and handling.

8.1.7 Reagents should be made in quantities to minimize waste. The shelf life of color test reagents is two years.

8.2 Color Tests and Reagents:

The following lists the commonly used color test reagents and some examples of reactions with various drugs. The references for each test are in parenthesis.

8.2.1 Bates Test (8.3.5) tests for cocaine base.

8.2.1.1 Procedure: The Bates test is used as the second part of the Cobalt thiocyanate test. If the Cobalt thiocyanate test is negative, add Marquis reagent to spot well.

8.2.1.2 Results: The formation of a very blue precipitate indicates cocaine base, other compounds give weaker blue or no reaction.

8.2.2 Benedict's Solution (8.3.2) tests for reducing sugars and some antibiotics.

8.2.2.1 Recipe: 1.73 g of copper sulfate in 10 mL of water. With the aid of heat, dissolve 17.3 g trisodium citrate and 10 g of anhydrous sodium carbonate in 80 mL of H_2O. Pour the two solutions together and let cool. Dilute to 100 mL with water.

8.2.2.2 Procedure: Add 0.5 mL of the reagent to sample and heat.

8.2.2.3 Results:

- Ascorbic acid, strong reducing agents, glucose, tetracycline – red
- Streptomycin - orange/brown

8.2.3 Chen's Test (8.3.2) tests for phenethylamines.

8.2.3.1 Recipe: 1 g copper sulfate and 1 mL glacial acetic acid in 100 mL H_2O.

8.2.3.2 Procedure: Make an approximate 1% aqueous solution of the sample, add equal volumes of Chen's reagent and 2N NaOH.

8.2.3.3 Results: ephedrine, PPA and pseudoephedrine – purple

8.2.4 Cobalt Thiocyanate reacts with tertiary and quaternary amines to form a blue precipitate and used for general screening. May be used in conjunction with the Bates test (8.2.1) or the Stannous Chloride test (8.2.23).

8.2.4.1 Recipes:

- 2 g cobalt thiocyanate in 100 mL H_2O or methanol (8.3.1)
- 2 g cobalt thiocyanate in 100 mL H_2O and 100 mL of glycerine. (8.3.3)
- 1.4 g $CoCl_2$. $6H_2O$ and 0.9 g NH_4SCN in 100 mL H_2O. (8.3.7)

8.2.4.2 Procedure: Place reagent in well and add sample.

8.2.4.3 Results:

- Cocaine HCl – blue precipitate forms, cocaine base may be initially negative or faintly blue, but blue intensifies upon the addition of dilute HCl.
- PCP - blue
- Amitriptyline / doxepin - blue
- barbiturates with unsaturated side chain (i.e. butalbital) - faint blue

8.2.5 Dille - Koppanyi Test (8.3.9) reacts with barbiturates.

8.2.5.1 Recipe:

- DK1: 0.1 g cobaltous acetate tetrahydrate in 100 mL methanol plus 0.2 mL glacial acetic acid
- DK2: 5 mL isopropyl amine in 95 mL methanol

8.2.5.2 Procedure: This is a two part test. Place 2 drops of DK1 reagent in a well. Add sample. Add 1 drop of DK2 reagent. When doing multiple samples, they should be separated to avoid cross-contamination due to reagent spreading.

8.2.5.3 Results:

- barbiturates - blue purple
- theophylline, glutethimide and hydantoins - purple
- ampicillin - brown

8.2.6 Duquenois - Levine Test (8.3.3, 8.3.4) reacts with marijuana and hash oil.

8.2.6.1 Recipe: 4 g vanillin and 2.5 mL fresh acetaldehyde per 200 mL 95% ethanol

8.2.6.2 Procedure: See Marijuana section.

8.2.6.3 Results: marijuana/hash oil – blue/purple, pink/purple extracts into $CHCl_3$

8.2.7 Ehrlich's Reagent (8.3.7) reacts with indole moiety and some amines.

8.2.7.1 Recipe: 5 g p-dimethylaminobenzaldehyde to 50 mL of 95% ethanol and 50 mL of conc. HCl

8.2.7.2 Procedure: Place reagent in well and add sample.

8.2.7.3 Results:

- LSD, psilocyn - purple (beware of leaching of dyes in blotter paper or tablets)
- benzocaine, procaine - yellow

8.2.8 Fehlings Solution (8.3.8) reacts with reducing compounds such as sugars.

8.2.8.1 Recipe:

- Fehlings1 - 3.46 g copper sulfate per 50 mL H_2O
- Fehlings2 - 86.5 g sodium potassium tartrate and 35 g of NaOH per 250 mL of H_2O

8.2.8.2 Procedure: Dissolve sample in water and mix. Add 5 drops of Fehlings1 and 5 drops of Fehlings2 and mix. Heat on steambath for approximaterly 5 minutes or until warm.

8.2.8.3 Results: reducing sugars – yellow to red.

8.2.9 Ferric Chloride ($FeCl_3$) tests for phenols and GHB.

8.2.9.1 Recipes:

- 9% aqueous solution (8.3.14)
- 5% aqueous solution (8.3.2)

8.2.9.2 Procedure: Place sample into a solution of water or methanol and add a drop of reagent.

8.2.9.3 Results:

- salicylamide - dark purple
- acetaminophen - blue
- hydrolyzed aspirin – purple (to hydrolyze a sample, place in H_2O, add a little acid and heat)
- GHB – red/brown

8.2.10 Fiegel's / Nitroprusside (nitroferricyanide) (8.3.3, 8.3.8) for secondary amines.

8.2.10.1 Recipe: 1 g of sodium nitroprusside in 100 mL H_2O and 10 mL acetaldehyde

8.2.10.2 Procedure: Dissolve sample in 2N Na_2CO_3 and add reagent.

8.2.10.3 Storage: store in brown bottle and refrigerate.

8.2.10.4 Results: secondary amines - deep blue color

8.2.11 Froehdes (8.3.1, 8.3.2) reacts with narcotics and used for general screening.

8.2.11.1 Recipe: 0.5 g ammonium molybdate per 100 mL H_2SO_4 (conc.)

8.2.11.2 Procedure: Place reagent in well and add sample.

8.2.11.3 Results:

- heroin - purple → green
- codeine - green → red/brown
- morphine - deep purple → slate
- aspirin - blue → purple
- phenoxymethylpenicillin - blue
- pentazocine – blue
- acetaminophen – pale blue

8.2.12 GHB Color Test #3 (Smith Test) (8.3.13) for GHB powders and solutions. This test will not react with GBL or 1,4-butanediol.

8.2.12.1 Recipe:

- Bromocresol Green – 0.03 g bromocresol green in 100 mL of 4:1 methanol:DI water adjusting the pH to 7.0 with 0.1 sodium hydroxide
- Methyl Orange – 0.01 g methyl orange in 100 mL DI water adjusting the pH to 7.0 with 0.1 N sodium hydroxide
- Modified Schweppes Reagent: Mix solutions A and B
  - Solution A – 2 g dextrose in 20 mL of DI water
  - Solution B – 2.4 g aniline hydrochloride in 20mL methanol
- Mix Bromocresol Green and Methyl Orange together in a 1:1 ratio. Then, mix that indicator mixture with the modified Schweppes reagent in a 3:1 ratio.

8.2.12.2 Procedure: Add 0.5 mL of a liquid sample or a small amount of powder to a test tube. Add 2 drops of the mixed reagent and gently swirl.

8.2.12.3 Results:

- GHB – immediate green color
- Negative results – pinkish orange (generally the same or slightly darker than the original test solution)

8.2.13 Marquis (8.3.1, 8.3.2) reacts with opiates and phenethylamines and is used for general screening.

8.2.13.1 Recipe:

- 10 mL 37% formaldehyde in 90 mL H_2SO_4 (conc.)
- 2 mL 37% formaldehyde in 75 mL H_2SO_4 (conc.)

8.2.13.2 Procedure: Place reagent in well and add sample.

8.2.13.3 Storage: Keep tightly capped.

8.2.13.4 Results:

- opiates - purple
- amphetamine/methamphetamine - orange/brown
- aspirin – pink → deep red on standing
- phenoxymethylpenicillin - red
- MDA/MDMA - black

8.2.14 Mayer's Reagent (8.3.7) reacts with alkaloids

8.2.14.1 Recipe: Dissolve 1 g of mercuric chloride in 100 mL H_2O. Add solid potassium iodide until the reddish precipitate first formed dissolves. Reagent should be clear and pale yellow in color.

8.2.14.2 Procedure: Add 0.1 N HCl to a test tube. Add sample to acid and mix. Add Mayer's reagent to the acid solution.

8.2.14.3 Results: alkaloids – a white to yellow precipitate is formed

8.2.15 Meckes (8.3.1, 8.3.2) reacts with narcotics and used for general screening.

8.2.15.1 Recipe: 1 g selenious acid per 100 mL H_2SO_4 (conc.)

8.2.15.2 Procedure: Add reagent to well then sample.

8.2.15.3 Results:

- heroin - green/blue
- codeine - bright-green/blue green
- PCP - light yellow
- quinine - light yellow

8.2.16 Methylene Blue (8.3.10) reacts with vitamin C.

8.2.16.1 Recipe: 12.5 mg of methylene blue dissolved in 25 mL of 95% ethanol.

8.2.16.2 Procedure: Add reagent to well and add sample. It may be helpful to run a blank to compare the results.

8.2.16.3 Results: Vitamin C - slowly decolorizes solution from dark blue to light blue.

8.2.17 Nitric Acid (HNO_3) (8.3.1, 8.3.2) reacts with opiates and mescaline.

8.2.17.1 Recipe: concentrated nitric acid

8.2.17.2 Procedure: Place reagent in well and add sample.

8.2.17.3 Results:

- heroin - yellow green
- morphine - red
- codeine - orange
- mescaline - red
- acetaminophen – fumes, orange brown

8.2.18 Parri (8.3.11) reacts with barbiturates.

8.2.18.1 Recipe: cobaltous acetate (solid), barium oxide (solid), and methanol

8.2.18.2 Procedure: Mix cobaltous acetate, BaO and powdered sample in equal parts in a spot plate well, add methanol.

8.2.18.3 Results: barbiturates - blue

8.2.19 Scotts - Modified Cobalt Thiocyanate (8.3.3) reacts with cocaine.

8.2.19.1 Recipe: 2 g cobalt thiocyanate in 100 mL H_2O and 100 mL of glycerine.

8.2.19.2 Procedure: Add reagent to well or tube and add sample. Dissolve the blue precipitate from the $Co(SCN)_2$ by the addition of HCl. Add $CHCl_3$

8.2.19.3 Results: cocaine - blue color in the lower ($CHCl_3$) layer.

8.2.20 Silver Nitrate (8.3.15) indicates the presence of chloride ions.

8.2.20.1 Recipe: 5.0% w/v solution of silver nitrate in DI water.

8.2.20.2 Caution: Poison; will cause staining.

8.2.20.3 Storage: Store in the refrigerator in a dark environment.

8.2.20.4 Procedure: Dissolve sample in water. Add silver nitrate solution. A white, curdy precipitate will form in the presence of chloride ions which will be insoluble in nitric acid. The precipitate will be soluble in 6N ammonium hydroxide.

8.2.21 Stannous Chloride modification for $Co(SCN)_2$ - HCl acidified (8.3.9) differentiates between “caines”.

8.2.21.1 Recipe: 5 g $SnCl_2$ and 10 mL conc. HCl diluted to 100 mL with H_2O

8.2.21.2 Procedure: The Stannous Chloride test is used as the second part of the Cobalt thiocyanate test. After performing the cobalt thiocyanate test, add a drop of stannous chloride reagent.

8.2.21.3 Results:

- Cocaine salts - blue remains
- Cocaine base - blue color forms (initially negative)
- Other compounds which turned blue initially - blue fades

8.2.22 Sulfuric Acid (H_2SO_4) (8.3.1, 8.7.2)

8.2.22.1 Recipe: concentrated sulfuric acid

8.2.22.2 Procedure: Add reagent to well and add sample.

8.2.22.3 Results:

- tetracycline - purple turning to yellow upon addition of water
- 2,3-MDMA, 2,3-MDA – rose
- 3,4-MDMA, 3,4-MDA – gray-brown

8.2.23 Tannic Acid (8.3.3) reacts with xanthines.

8.2.23.1 Recipe: 1% aqueous solution of tannic acid

8.2.23.2 Procedure: Add reagent to test tube then add powdered sample.

8.2.23.3 Results: caffeine and theophylline - positive test will produce a precipitate which develops from "streamers" immediately visible in the solution.

8.2.24 TBPEE Solution (8.3.8) differentiates between amines.

8.2.24.1 Recipe:

- 0.01g Tetrabromophenolphthalein ethyl ester (TBPEE) in 100 mL CCl_4
- 10.6 g sodium carbonate in 100 mL H_2O (2N solution)

8.2.24.2 Caution: Carbon tetrachloride is carcinogenic. Use appropriate safety precautions.

8.2.24.3 Procedure: Dissolve suspected amine in 2N Na_2CO_3 solution and add TBPEE solution. Note color change in the bottom TBPEE layer.

8.2.24.4 Results:

- primary amines - violet
- secondary amine - blue
- tertiary amine – red

8.2.25 Van Urk's (8.3.12) reacts with the indole moiety and some amines.

8.2.25.1 Recipe: 125 mg p-dimethylaminobenzaldehyde, 65 mL of concentrated H_2SO_4, and 2 drops of ferric chloride (USP T. S.) diluted to 100 mL with distilled water.

8.2.25.2 Procedure: Add reagent to well then add sample.

8.2.25.3 Results: LSD – blue/purple

8.2.26 Weber Test (8.3.6) reacts with psilocyn.

8.2.26.1 Recipe: 0.01 g of Fast Blue B or Fast Blue BB in 10 mL H_2O

8.2.26.2 Procedure: Add 2 to 3 drops of reagent to a sample of mushrooms. Observe slow color change. Add 1 to 2 drops of conc. HCl, observe color change.

8.2.26.3 Results: Psilocyn – Initially the solution turns red. The solution will turn from red to blue when the acid is added.

8.3 References:

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